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Nitrate Reduction Potential in Clear-cut Scots Pine (*Pinus sylvestris* L.) Forest Soil

TORBJÖRN LINDBERG and LEIF KLEMEDTSSON

Department of Microbiology

Abstract. Nitrate, formed as a consequence of clear-cutting, may be lost from the ecosystem by denitrification. The present study was undertaken to investigate whether humus from a clear-cut coniferous forest could support nitrate reduction. Humus soil was collected 16 months after clear-cutting, sieved and incubated in the laboratory at different temperature and moisture conditions after addition of NH_4^+–N and NO_3^-–N. Production of N_2O–N was measured in the absence and presence of acetylene (C_2H_2).

Incubation under simulated field conditions optimal for denitrification (51 or 91% of maximum water holding capacity at 15°C) and in the presence of up to 100 ppm NO_3^-–N (43–83 times that *in situ*) or with 1 000 ppm NH_4^+–N (20–27 times that *in situ*), produced no N_2O. Ammonium was immobilized but not nitrified. N_2O production could be detected when humus was incubated at 24°C, at maximum water holding capacity and in the presence of 400 ppm NO_3^-–N. The main product of denitrification was N_2O. No nitrate was assimilated or reduced to ammonium.

The maximum rates of N_2O production in the presence of C_2H_2 for humus from plots with and without slash were 0.39 and 0.40 μg N_2O–N g^{-1} h^{-1}, respectively. It is unlikely that nitrate reduction would occur under *in situ* conditions but a potential exists under high moisture and temperature conditions in the presence of large amounts of nitrate.

INTRODUCTION

The reduction of inorganic nitrogen, primarily nitrate, to nitrous oxide (N_2O) and dinitrogen, is one of the most important but least quantified processes causing nitrogen loss from soil. Nitrate reduction can occur either chemically (chemo-denitrification or chemical nitrate reduction; Knowles, 1981), or by the action of bacteria using nitrate as an electron acceptor under anaerobic conditions (Payne, 1981). Nitrogen in the form of N_2O can also be lost from the soil system during nitrification (Bremner & Blackmer, 1981).

In undisturbed coniferous forests soils, NO_3^- is normally present only in low concentration; this and the fact that these soils are well aerated, has led to a general belief that nitrate reduction seldom occurs in such soils (Tamm et al., 1974). This is also supported by the few investigations made on acidic podsolic soils (Overrein, 1969; Nömmik & Thorin, 1972; Mahendrappa, 1974).

After clear-cutting a coniferous forest substantial amounts of easily decomposed carbon, mainly in the form of dead roots, are released. Increased mineralization and absence of root-uptake leads to accumulation of NH_4^+ which can be nitrified and further denitrified (Tamm et al., 1974; Vitousek & Melillo, 1979; Bormann & Likens, 1979). Melillo et al. (1983) reported higher potential denitrification in a two-year-old clear-cut (0.4–51.6 kg N ha^{-1} yr^{-1}) than in a 50-year-old northern hardwood forest stand (1.4–9.0 kg N ha^{-1} yr^{-1}) growing on an acid soil. The present study was undertaken to investigate whether humus from a clear-cut coniferous forest could support nitrate reduction.

MATERIALS AND METHODS

The clearcut site Ivantjärnsheden (Ih 0) is one of the sites within the Swedish Coniferous Forest Project (SWECON). The soil texture is glacifluvial sand and the soil profile is a weak iron podsol. The mature vegetation before clear-cutting was a Scots pine (*Pinus sylvestris* L.) forest. A full description of the SWECON research sites is given by Axelsson & Bråkenhielm (1980). The site (2 ha) was clear-cut in March 1976 and the slash was removed from half of the plots and left in double amounts on the remaining plots.

Soil from the humus-layer (A_{01}/A_{02}) in the clear-cut area was collected from plots with and without slash in August 1977 (16 months after clear-cutting) and passed through a 2 mm sieve. Some properties of the sieved soil are shown in Table 1. The soil was stored at 2°C until the experiments were performed.

Experiment 1

The experiment was designed to determine whether a potential for nitrate reduction existed under opti-

Table 1. *Properties of sieved humus used in the experiments*

Properties	Soil from areas with slash	Soil from areas without slash
Maximum water holding capacity (% H_2O dry weight^{-1})	260	474
Total-N (%)	ND[b]	0.81
NH_4^+–N (µg g dry weight^{-1})[a]	ND (51)	247 (37)
NO_3^-–N (µg g dry weight^{-1})[a]	ND (1.2)	0.2 (2.3)
Organic matter (%)	44	75
pH	4.3	4.3

[a] Amounts of NH_4^+–N and NO_3^-–N at the start of the experiments. The corresponding values for soil at time of collection are shown within parenthesis.
[b] ND: not determined.

mal field conditions of temperature, moisture and nitrate concentrations. An analysis of abiotic and chemical data collected within SWECON for the site showed that the maximal soil temperature was 15°C, the maximal soil moisture in undisturbed humus-layer was 257% H_2O on a dry weight (dw) basis and the maximal amounts NO_3^-–N and NH_4^+–N 3 µg/g dw (ppm) and 100 µg/g dw (ppm), respectively. The soil was incubated at 15°C and 243% H_2O dw^{-1} (51 or 91% of maximum water holding capacity; MWHC; Table 1) with different additions of NO_3^-–N (3, 10, 50, 100 ppm) or with addition of NH_4^+–N (1 000 ppm).

Experiment 2

The soil was incubated at maximum water holding capacity at 24°C with and without the addition of 400 ppm NO_3^-–N.

Denitrification was measured using the acetylene inhibition method (Yoshinari & Knowles, 1976; Yoshinari et al., 1977). The principle of the method is that acetylene (C_2H_2) blocks nitrous oxide reductase, meaning that N_2O becomes the sole gaseous product of denitrification. At the start of the experiments soil moisture was adjusted with distilled water and the soil was preincubated at the experimental temperature for 24 hours. Ten grams of soil were placed into six 100 ml glass bottles and nitrogen salt solutions or water were added. The bottles were immediately sealed with butyl rubber stoppers. Acetylene (10% v/v) was added to each of triplicate bottles, and an equal amount of air was added to another three bottles. The atmosphere to soil ratio in the bottles was approximately 3 to 1. Preliminary studies showed that CO_2 production in the sealed bottles was linear for at least 100 hours but for less than 200 hours (Fig. 1 A, B). Therefore, in subsequent experiments the bottles were analysed every 100 hours for the production of N_2O and CO_2. After each analysis the bottles were flushed and new acetylene/air was added. This was repeated up to 1 800 hours or until no further N_2O production could be detected. Nitrate and ammonium content in the soil was determined after termination of the experiments (Popovic, 1980).

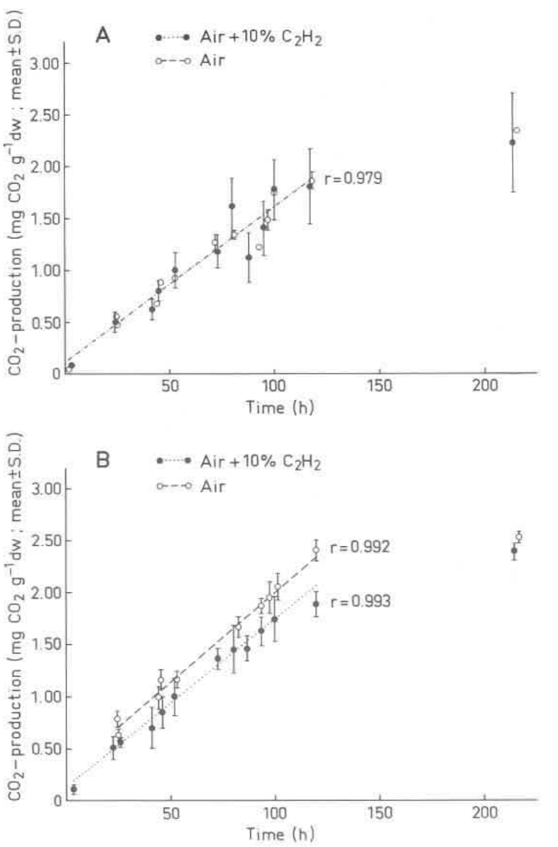

Fig. 1. CO_2-production in sealed bottles with soil from slash (A) or no slash (B) plots. n=3.

Gas samples were withdrawn from the bottles, using a gas-tight syringe, and analysed immediately for N_2O and CO_2 by gas chromatography as described by Klemedtsson et al. (1977). Correction for N_2O dissolved in the soil water phase was made using Henry's law (Flett et al., 1976).

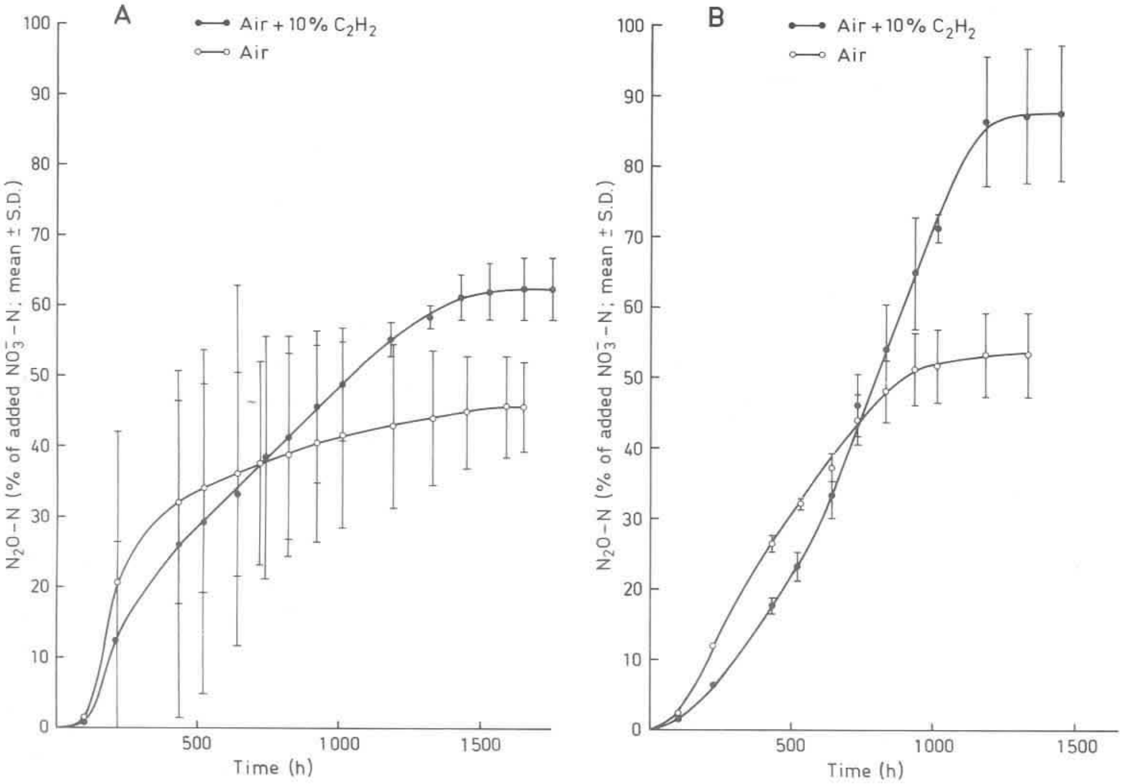

Fig. 2. N_2O-production in bottles with soil from slash (A) or no slash (B) plots. The soil was incubated at maximum water holding capacity at 24°C after addition of 400 ppm NO_3^-–N. The bottles were flushed every 100 h. $n=3$.

RESULTS

Incubation of humus under simulated optimal field conditions for denitrification and with up to 100 ppm NO_3^-–N or with 1 000 ppm NH_4^+–N produced no N_2O. This first experiment was terminated after 230 hours of incubation.

Nitrous oxide production could be detected when the humus was at MWHC and the temperature was 24°C and only in the presence of large amounts of nitrate (400 ppm; Fig. 2 A, B). In this case N_2O was produced both in the presence and absence of acetylene. Initially almost similar amounts of N_2O were produced with or without C_2H_2, which is an indication that N_2O was the main product of denitrification. When approximately 140–180 ppm N_2O (corresponding to 35–45% of the added NO_3^-–N) had been produced, N_2O production without C_2H_2 ceased while it continued in C_2H_2-amended bottles, thus N_2 gradually became the dominant end product in the absence of C_2H_2. After 1 500 hours N_2O production had ceased completely. The N_2O–N recovered was between 63 and 88% of added NO_3^- in the presence of C_2H_2 and between 46 and 53% in the absence of C_2H_2. The total recovery of added nitrogen as N_2O–N plus remaining NO_3^-–N was 75–84% in the absence of C_2H_2 and 104–111% in the presence of C_2H_2 (Table 2). The NO_3^-–N not accounted for in the absence of C_2H_2 was probably reduced to N_2.

Yeomans & Beauchamp (1978) have shown that the acetylene block may only hold for a limited period of time (in their case 168 hours) and then N_2O disappears from the system. In our case soil samples were almost constantly exposed to C_2H_2 for at least 1 500 hours, but samples with C_2H_2 still produced more N_2O than samples without C_2H_2, suggesting that the block was still functional. This is further indicatated by the complete recovery of added nitrogen in samples with C_2H_2 (Table 2), which also means that no nitrate was immobilized or reduced to NH_4^+ (Caskey & Tiedje, 1979, 1980).

Ammonium (1 000 µg NH_4^+–N/g dw) was added

Table 2. *Nitrogen balance for soil incubated at 24°C, for 1400–1800 hours at maximum water holding capacity. n=3*

N-fraction	Soil without slash		Soil with slash	
	With C_2H_2	Without C_2H_2	With C_2H_2	Without C_2H_2
NO_3^-–N added (ppm)	400	400	400	400
N_2O–N found (ppm)	350	213	250	182
NO_3^-–N found (ppm)	95	121	166	116
% N_2O–N found of added NO_3^-–N	88	53	63	46
% N_2O–N plus NO_3^-–N found of added NO_3^-–N	111	84	104	75

to check the nitrifying capacity of the soil. After 64 days of incubation only 2.8 and 2.5 μg NO_3^-–N/g dw and 271 and 301 μg NH_4^+–N/g dw could be detected in the absence and presence of C_2H_2, respectively. Since nitrification will not occur in the presence of 10% C_2H_2 (Berg et al., 1982) the disappearance of ammonium in C_2H_2-amended bottles showed the high immobilization capacity of the soil. No nitrification occurred in bottles without C_2H_2 and ammonium disappearance was the same as in the presence of C_2H_2. No N_2O was detected in this experiment. CO_2 production rates decreased slightly during incubation (Fig. 3 A, B), possibly due to energy exhaustion. The presence of C_2H_2 lowered that CO_2 production rate, which is contrary to what others have found (Klemedtsson et al., 1977; Ryden et al., 1979). However, the effects of C_2H_2 on carbon metabolism may vary with nitrate content (Haider et al., 1983). Our results indicate that long-term exposure to C_2H_2 may inhibit microorganisms.

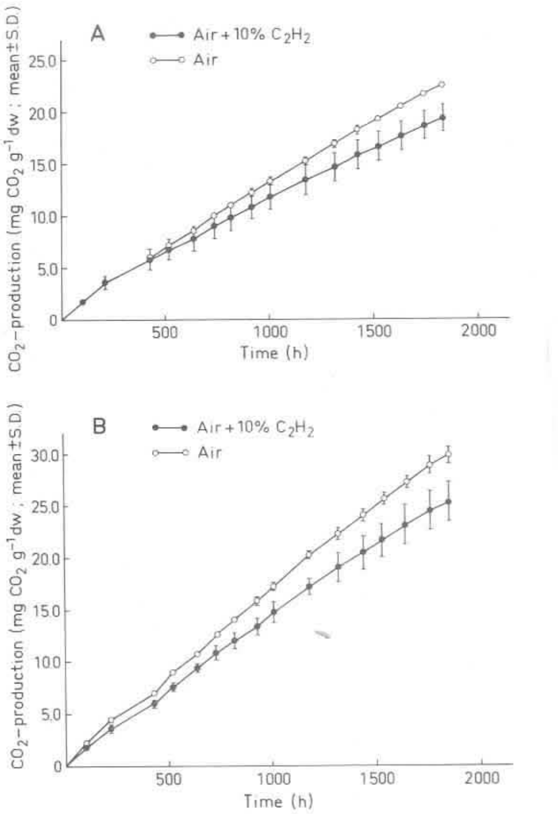

Fig. 3. CO_2-production in bottles with soil from slash (A) or no slash (B) plots. The soil was incubated at maximum water holding capacity at 24°C after additions of 400 ppm NO_3^-–N. The bottles were flushed every 100 h. *n*=3.

In general, the results were similar for humus with and without slash removal. However, less CO_2 and N_2O were produced from areas with slash, possibly due to lower organic matter content (Table 1), which was not an effect of the slash itself, but of some mineral soil accidentally present in the sampled humus layer. Variations in N_2O production were large between replicates and no conclusions can be made concerning the differences in nitrate reduction potential.

DISCUSSION

The factors that might affect denitrification rates in our experiments are temperature, moisture and amount of nitrate. The amount of nitrate used in both of our experiments was higher than 40 ppm, an amount others have found causes zero-order denitrification (Knowles, 1981). The Q_{10} of denitrification is usually around 2 at the temperatures used in our experiments (Knowles, 1981). Higher temperatures lower the oxygen content of soil because of higher respiration rates which might stimulate denitrification (Smith & Tiedje, 1979*b*). Knowles (1981) stated that the lower limit for denitrification is around 60% of MWHC. We incubated humus from the no-slash plots at 51% of MWHC and humus from the slash plots at 91% of MWHC and found no nitrate reduction (Experiment 1). It is possible that the porous humus-layer in this kind of forest soil has a higher pO_2 than many other soils and that the combination of waterlogging and temperature increase created enough anaerobiosis for denitrification to occur in Experiment 2.

The main gaseous product was N_2O, as has been found for other soils with low pH (Nömmik 1956; Blackmer & Bremner, 1978). An explanation was offered by Blackmer & Bremner (1978; 1979), who stated that the reduction of N_2O to N_2 is inhibited by large amounts of NO_3^- and that the inhibitory effect increases at lower pH.

Müller et al. (1980) reported denitrification values of 0.12–0.16 $\mu g\ N\ cm^{-3}\ day^{-1}$ for the organic later of a podzolized Finnish Scots pine forest soil amended with nitrate and incubated anaerobically. Our recalculated maximum rates are 2.14–3.92 $\mu g\ N_2O\text{–}N\ cm^{-3}\ day^{-1}$. However, the Finnish group found even higher rates in the mineral soil (B) horizon, with 14.4–17.8 $\mu g\ N\ cm^{-3}\ day^{-1}$ of nitrate reduction. Nömmik & Thorin (1972) reported low denitrification in undisturbed forest raw humus (pH 4.3) with, at the most, 24 $\mu g\ NO_3^-\text{–}N$ of an added 400 $\mu g\ NO_3^-\text{–}N/g$ dw recovered as gaseous nitrogen after 10 days' anaerobic incubation at 20°C. In our second experiment, 29–90 μg of an added 400 $\mu g\ NO_3^-\text{–}N$ was recovered as $N_2O\text{–}N$ during the first ten days.

The maximum rates of nitrous oxide production found in our experiments are lower than those reported for arable soils using the same method. Klemedtsson et al. (1977) reported a V_{max} of 2.3 $\mu g\ N_2O\text{–}N\ g^{-1}\ h^{-1}$ for an anaerobic waterlogged soil, Yoshinari et al. (1977) 1.5 $\mu g\ N_2O\text{–}N\ g^{-1}\ h^{-1}$ for an anaerobic moist soil and Ryden et al. (1979) a maximum of 1.1 $\mu g\ N_2O\text{–}N\ g^{-1}\ h^{-1}$ for soil slurries in an atmosphere of 20% oxygen and 80% argon. Our rates for humus soil with and without slash were 0.39 and 0.40 $\mu g\ N_2O\text{–}N\ g^{-1}\ h^{-1}$ produced respectively, in the presence of C_2H_2. However, these rates are difficult to compare since different sample treatment such as removal of roots, storage and changing moisture, affect denitrification (Patten et al., 1980; Smith & Tiedje, 1979 *a*). In our experiments it took up to 500 hours (21 days) to reach the maximum N_2O-production rate. Klemedtsson et al. (1977) achieved maximum N_2O-production less than 5 days after adding 40 $\mu g\ NO_3^-\text{–}N/g$ dw to a Swedish arable soil, and Müller et al. (1980) less than 150 hours after addition of 42 $\mu g\ N\ cm^{-3}$ to a Finnish forest soil. We cannot offer an explanation for this very long accelerating phase. The possibility of abiotic nitrate reduction was not investigated, but Nömmik & Thorin (1972) found no reduction of NO_3^- in sterilized soil similar to the one used in our experiments.

Humus without added nitrate or with up to 100 ppm $NO_3^-\text{–}N$ showed no denitrification. The amounts of nitrate, ammonium and soil pH were measured biweekly at the site after clear-cutting. Contrary to what has often been found after clear-cutting, there was no change in soil pH or in the nitrate concentrations, that are normally less than 3 ppm. However, in 1977 (the year after clear-cutting), amounts of NH_4^+ averaged 3.7 times greater in the clear-cut than in a nearby forested site, and measured up to 100 ppm the humus (T. Lindberg & B. Popovic, unpublished). Four years after clear-cutting there still had been no change in pH or nitrate content in the soil, while the ammonium content was still increasing. Production and leaching of nitrate may vary greatly in different clear-cut sites in Sweden. It may occur as soon as one year after cutting but may be delayed 10 years, especially on poor sites (Wiklander, 1981). Our results show that it is unlikely that nitrate reduction would occur in the investigated humus-layer under *in situ* conditions. However, denitrification could potentially occur in a clear-cut of nitrification is also occurring.

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Torbjörn Lindberg
Department of Microbiology
S-750 07 UPPSALA, Sweden

Leif Klemedtsson
Department of Microbiology
S-750 07 UPPSALA, Sweden